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A micro-architecturally biomimetic collagen template for mesenchymal condensation based cartilage regeneration

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ABSTRACT

The unique arcade-like orientation of collagen fibers enables cartilage to bear mechanical loads. In this study continuous-length aligned collagen threads were woven to emulate the interdigitated arcade structure of the cartilage. The weaving pattern provided a macropore network within which micromass cell pellets were seeded to take advantage of mesenchymal condensation driven chondrogenesis. Compression tests showed that the baseline scaffold had a modulus of 0.83 ± 0.39 MPa at a porosity of 80%. The modulus of pellet seeded scaffolds increased by 60% to 1.33 ± 0.37 MPa after 28 days of culture, converging to the modulus of the native cartilage. The scaffolds displayed duress under displacement controlled low-cycle fatigue at 15% strain amplitude such that load reduction stabilized at 8% after 4500 cycles of loading. The woven structure demonstrated a substantial elastic recoil where 40% mechanical strain was close to completely recovered following unloading. A robust chondrogenesis was observed as evidenced by positive staining for GAGs and type II collagen and aggrecan. Dimethyl methylene blue and sircol assays showed GAGs and collagen productions to increase from 3.36 ± 1.24 and 31.46 ± 3.22 at day 3 to 56.61 ± 12.12 and 136.70 ± 12.29 μg/μg of DNA at day 28 of culture. This woven collagen scaffold holds a significant potential for cartilage regeneration with shorter *in vitro* culture periods due to functionally sufficient mechanical robustness at the baseline. In conclusion, the mimicry of cartilage's arcade architecture resulted in substantial improvement of mechanical function while enabling one of the first pellet delivery platforms enabled by a macroporous network.

Statement of significance

Mesenchymal condensation is critical for driving chondrogenesis, making high density cell seeding a standard in cartilage tissue engineering. Efforts to date have utilized scaffold free delivery of MSCs in pellet form. This study developed a macroporous scaffold that is fabricated by weaving highly aligned collagen threads. The scaffold can deliver high density cell condensates while providing mechanical stiffness comparable to that of cartilage. The scaffold also mimicked the arcade-like orientation of collagen fibers in cartilage. A highly robust chondrogenesis was observed in this mesenchymal cell pellet delivery system. Baseline mechanical robustness of this scaffold system will enable delivery of cell pellets as early as three days.

1. Introduction

Cartilage is a load-bearing tissue which facilitates low-friction articulation in diarthrodial joints. Decades of duty cycles and limited self-regeneration capacity take their toll on cartilage integrity, resulting in the formation of defects [1]. Cartilage defects are repaired by microfracturing, mosaicplasty or chondrocyte transplantation. Microfracturing results in mechanically inferior fibrocartilage formation [2]. Mosaicplasty may result in donor site

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[1] Prof. Victor M. Goldberg passed away on February 7, 2015 before he was able to review this manuscript. He has contributed intellectually to the design of experiments and the interpretation of data. He was a co-author in several conference abstracts which were related to this manuscript. We dedicate this paper to his memory and long standing service and leadership in orthopaedic research. He will be missed greatly.

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morbidity and healing seams [3]. Chondrocyte transplantation is limited to the repair of small defects and clinical trials report mixed outcomes [4,5]. Therefore, regenerative medicine approaches are needed to restore functionality of damaged cartilage.

Chondrocytes and stem cells from various sources (marrow, adipose, embryonic, *etc.*) have been used in cartilage TE. While chondrocytes perform better than mesenchymal stem cells in terms of generating a cartilage-like matrix [6,7], finding sufficient donor tissue and maintaining the chondrocytic phenotype during *in vitro* expansion remain as challenges [8–10]. The challenge with mesenchymal stem cells (MSCs) has been the attainment of stable chondrogenic differentiation [11]. What appears to induce chondrogenesis of MSCs relatively reliably is high density cell seeding [12,13] during which MSCs undergo condensation and cell–cell adhesions are conducive to chondrogenesis. Formation of neocartilage during embryogenesis also occurs through a similar mesenchymal condensation process [14,15]. While such scaffold free strategies have met with some success; weak mechanical properties, extended culture time [16] and demanding bioreactor strategies to provide strength and shape to the high density condensate increase the cost and time [17].

Since condensation is critical to the attainment of chondrogenesis, a scaffold system which enables the delivery of MSCs in pellet form would unify the chondrogenic merit of condensation with the structural merits of scaffolds. Cell pellets, at sufficient numbers for appropriate condensation [16], are sized at hundreds of microns to a millimeter. Therefore, a scaffold that can deliver high density condensates need to be macroporous. Attaining sufficient mechanics while accommodating high levels of ordered porosity requires a special material, with a sophisticated microstructure. To the best of our knowledge, there have been no reports of scaffold systems designed to deliver cell pellets.

Electrochemical processing of collagen solutions addresses the poor mechanics of collagen products by compacting and aligning collagen solutions as threads whose strength is among the strongest of collagen biomaterials (50–100 MPa) [18–21]. Previous work used such high strength collagen threads to fabricate woven scaffolds for tendon and ligament tissue engineering [22,23]. Weaving patterns of this scaffold and other woven scaffolds [24] do not entail macroscale porosity; thus, they do not allow for pellet seeding.

In this study, a macroporous scaffold was woven from electrocompacted collagen threads to accommodate cell-pellets. The weaving pattern mimicked some of the architectural characteristics of the native arcade-like orientation patterns of collagen fibers in cartilage. In our strategy, the woven scaffold was purely a mechanical crucible for protecting and delivering cell pellets in mechanically robust environment. Chondrogenesis was attained by mesenchymal condensation and TGFβ-3. Combination of mesenchymal condensation driven chondrogenesis within a load-bearing scaffold as such may help expedite repair timeline in the future by enabling earlier implantation of the scaffold framework.

2. Materials and methods

2.1. Scaffold fabrication

Scaffold production is comprised of 3 steps. In the first step collagen thread is produced by electrochemical alignment process as explained in previous publications [22,25]. Briefly, acid solubilized collagen molecules (3 mg/mL) are dialyzed against deionized water. Following dialysis, the solution is applied between two stainless steel electrode wires (1.8 mm separation) in an electrochemical cell (Fig. 1a). Electrical currents generate a pH gradient in the electrochemical cell. Collagen molecules acquire positive charge near the cathode and negative charge near the anode. Electrorepulsion by electrodes compact the collagen molecules at the isoelectric point of the electrochemical cell where the net charges of the collagen molecules are zero. Our prior work also demonstrated that the longer axes of collagen molecules are oriented parallel to the longer axis of the thread [26]. We used this principle in the design of a device to fabricate the aligned collagen threads in continuous length [22]. The diameters of the threads were 0.11 ± .03 mm.

Uncrosslinked collagen threads were then woven in a zig–zag pattern around a set of equidistant pins with 1 mm diameter (Fig. 1b). The weaving pattern of the scaffold (Fig. 1c) mimicked the arcade structure of cartilage (Fig. 2a and b) such that the molecular orientation of collagen was parallel to the plane of the surface and became vertically oriented by mid thickness of the scaffold.

In the second step, two planar graphite electrodes were used to fabricate electrochemically compacted collagen sheets (Fig. 1c). One compacted collagen sheet was placed each on the top and the bottom of the woven pattern. These sheets served to stabilize the threads together.

In the third step, the threads and the sheets were crosslinked in genipin solution (0.625% w/v in 90/10 v/v ethanol and water mixed solution) for 72 h [19]. Following crosslinking, the pins were removed, leaving behind cylindrical pores of 1 mm diameter to be populated by cell pellets (Fig. 1d). Scaffolds were washed thoroughly with deionized water, dehydrated and stored at 4 °C until use. The nominal dimensions of resulting scaffolds were 4 × 2 × 1 mm in width, depth and height (Fig. 1c).

2.2. Polarized microscopy

Collagen fiber orientation in collagen threads and woven scaffold were visualized via polarized light optical microscope (Olympus BX51). An articular cartilage sample from lamb knee joint was utilized to compare the collagen fiber orientation thorough the thickness of cartilage and the woven scaffold.

2.3. Mechanical tests

2.3.1. Compressive mechanical properties

Woven scaffolds were prepared with dimensions of 4 mm × 2 mm × 1 mm and were soaked in 1× phosphate buffered saline (PBS) one hour before testing. Samples were subjected to constant strain-rate (1%/s) compression (Rheometrics Solid Analyzer RSAII, Rheometrics Inc., Piscataway, NJ, USA). The samples were highly resilient and did not fail; therefore, stress values reached by 10% strain are reported as maximum compressive stress. Apparent Young's modulus was determined by calculating the slope of stress–strain curve in the range of 3–6% strain by linear regression. The mean values were calculated from five samples (n = 5). Bovine and rabbit cartilage samples were cut from the distal femoral condyles using a 5 mm biopsy punch. Cartilage samples were also soaked in PBS about 1 h before compression tests and tested as described for collagen samples.

2.3.2. Fatigue test

Scaffolds were tested in cyclic mode using a servo-controlled fatigue test machine (Test Resources 800 L, Minnetonka, MN). Tests were performed under strain control up to 4500 cycles under a sinusoidal loading mode with frequency of 1 Hz and at a maximum strain value of 15%. This value is selected because it is in the natural strain range experienced by cartilage and is used in bioreactors for mechanical stimulation [27,28]. Under strain control, the load decreased with increasing number of cycles, with greater amplitude of reduction implying a greater degree of degradation of